

1                    PROCESS FOR THE PREPARATION OF AMPHIPHILIC  
2                    POLY(N-VINYL-2-PYRROLIDONE) BLOCK COPOLYMERS

3  
4                    FIELD OF THE INVENTION

5                    The invention relates generally to processes for  
6                    preparation of block copolymers; particularly to processes  
7                    for preparation of block copolymers by a two-step  
8                    polymerization and most particularly to processes for  
9                    preparing diblock and triblock copolymers comprising the  
10                   steps of: (a) performing radical polymerization of N-vinyl-2-  
11                   pyrrolidone in the presence of a radical initiator, a chain  
12                   transfer agent (optionally) and an alcoholic solvent to form  
13                   hydroxy-terminated poly(N-vinyl-2-pyrrolidone) and (b)  
14                   performing ionic polymerization of monomers or comonomers in  
15                   the presence of a catalyst or base and a macroinitiator  
16                   wherein said macroinitiator is the hydroxy-terminated poly(N-  
17                   vinyl-2-pyrrolidone) formed in step (a) thereby preparing said  
18                   diblock and triblock copolymers. Poly(N-  
19                   vinylpyrrolidone) formed in step (a) has a molecular weight  
20                   between 1,000 D and 700 kD and the diblock and triblock  
21                   copolymers have a molecular weight between 2,000 D and 700  
22                   kD.

## BACKGROUND OF THE INVENTION

The synthesis of well-defined polymers with controlled chain end functionalities is important for the achievement of nanotechnology. These polymers have been especially important as potential drug delivery vehicles. In the last decade, the use of various controlled polymerizations have resulted in well-defined copolymers with different designs. For example, nitroxide-mediated polymerization, dithio component-mediated reversible addition-fragmentation chain transfer and atom transfer radical polymerization (ATRP) are controlled processes, which offer control over molecular weight and molecular architecture (diblock, grafted or tapered copolymers). However, a few monomers such as vinyl acetate and N-vinyl-2-pyrrolidone (VP) do not form radicals stabilized by resonance and inductive effects, and therefore the polymerization of these monomers has not yet been performed efficiently by controlled radical polymerizations. Matyjaszewski *et al.* (Am. Chem. Soc. Symp. Ser. 685:258 1998 and J. Polym. Sci. Part A:Polym. Chem. 36:823-830 1998) reported the homopolymerization of VP using Me<sub>4</sub>Cyclam as a ligand. Chain end functionalities were difficult to obtain using the synthetic pathway described by Matyjaszewski *et al.*

The instant inventors are interested in functionalized

1 and well-defined poly(N-vinyl-2-pyrrolidone) (PVP) as a  
2 replacement for poly(ethylene glycol) (PEG) in diverse drug  
3 delivery systems. Although a number of diblock or triblock  
4 copolymers can form micelles in aqueous solution, few among  
5 them are truly suitable as drug carriers due to  
6 biocompatibility issues [Alexandridis *et al.* Current Opinion  
7 Colloid & Interface Science 2:478-489 1997; Rapoport *et al.* J  
8 Pharm. Sci. 91:157-170 2002; Kabanov *et al.* Adv. Drug Deliv.  
9 Rev. 54:223-233 2002; Nishiyama *et al.* Langmuir 15:377-383  
10 1999; Kakizawa *et al.* Langmuir 18:4539-4543 2002; Katayose *et*  
11 *al.* Bioconjugate Chem. 8:702-707 1997; Yamamoto *et al.* J.  
12 Controlled Release 82:359-371 2002; Liggins *et al.* Adv. Drug  
13 Deliv. Rev. 54:191-202 2002; Kim *et al.* J. Controlled Release  
14 72:191-202 2001; Yoo *et al.* J. Controlled Release 70:63-70  
15 2001; Luo *et al.* Bioconjugate Chem. 13:1259-1265 2002; Lim  
16 Soo *et al.* Langmuir 18:9996-10004 2002; Gref *et al.* Science  
17 263:1600-1603 1994 and Burt *et al.* Colloids Surf. B 16:161-  
18 171 1999]. Many studies have reported the use of polyester-  
19 block-poly(ethylene glycol) block copolymers [Yamamoto *et*  
20 *al.*; Liggins *et al.*; Kim *et al.*; Yoo *et al.*; Luo *et al.*; Lim  
21 Soo *et al.*; Gref *et al.* and Burt *et al.* journal citations,  
22 *supra*]. PEG is widely used as hydrophilic arm on the surface  
23 of nanoparticles [Kissel *et al.* Adv. Drug Deliv. Rev. 54:99-  
24 134 2002], liposomes[Gabizon *et al.* Adv. Drug Deliv. Rev.

24:337-344 1997]and polymeric micelles [Jones *et al.* Eur. J. Pharm. Biopharm. 48:101-111 1999; Kataoka *et al.* Adv. Drug Deliv. Rev. 47:113-131 2001 and Kabanov *et al.* Adv. Drug Deliv. Rev. 54:759-779 2002]. The PEG-based outer shell can actually prevent the nanocarrier uptake by the mononuclear phagocytic system via steric effects [ Jones *et al.*; Kataoka *et al.* and Kabanov *et al.* journal citations; *supra*]. This prevention substantially improves the circulation time of polymeric micelles in the blood stream. In cancer treatment, this prolonged time generally results in a selective accumulation in a solid tumor due to the enhanced permeability and retention effect of the vascular endothelia at the tumor site [Yokoyama *et al.* Cancer Res. 50:1693-1700 1990; Yokoyama *et al.* Cancer Res. 51:3229-3236 1991; Kwon *et al.* J. Controlled Release 29:17-23 1994; Yokoyama *et al.* J. Controlled Release 50:79-92 1998 and Yamamoto *et al.* J. Controlled Release 77:27-38 2001]. However, since aggregation of nanoparticles with PEG as corona occurs during lyophilization, it features some limitations. Thus, PEG is not ideally suited for efficient use in drug delivery systems.

Functionalized and well-defined PVP is an ideal component for replacement of PEG in drug delivery systems. PVP has been proven to be biocompatible [Haaf *et al.* Polymer

J. 17:143-152 1985]and has been extensively used in pharmaceutical industry. Particularly, PVP can be used as cryoprotectant [Doebbler et al. Cryobiology 3:2-11 1966] and lyoprotectant [Deluca et al. J. Parent. Sci. Technol. 42:190-199 1988]. Hence, replacing PEG with PVP in drug delivery systems might help to overcome some freeze drying problems.

Torchilin et al. [J. Microencapsulation 15:1-19 1998] pioneered the study of PVP as hydrophilic corona of liposomes. The design of polymeric micelles with PVP outer shell have presented promising features for pharmaceutical uses. Thus, Benahmed et al. [Pharm. Res. 18:323-328 2001] reported the preparation of PVP-based micelles consisting of degradable diblock copolymers. In the work of Benahmed et al. , PVP synthesis using 2-isopropoxyethanol as chain transfer agent was inspired from by previous work of Ranucci et al. [Macromol. Chem. Phys. 196:763-774 1995 and Macromol. Chem. Phys. 201:1219-1225 2000]. However, this synthetic procedure produced a lack of control over molecular weight, and did not quantitatively provide hydroxyl-terminated PVP , which is essential for polymerizing DL-lactide [Benahmed et al. Pharm Res. 18:323-328 2001]. Moreover, the removal of 2-isopropoxyethanol from the polymer turned out to be difficult because of its high boiling point (42-44°C at 13 mmHg) and its binding to PVP via hydrogen bonding [Haaf et al. Polymer J.

1 17:143-152 1985]. Alcohol entrapment into polymer might  
2 cause problems for subsequent reactions which require  
3 anhydrous and aprotic conditions such as the synthesis of  
4 poly(D,L-lactide). Sanner et al. [Proceeding of the  
5 International Symposium on Povidone, University of Kentucky:  
6 Lexington, KY, 1983, pp.20] reported the synthesis of  
7 hydroxyl-terminated PVP oligomers via free radical  
8 polymerization in isopropyl alcohol (IPA), using cumene  
9 hydroperoxide as an initiator. <sup>1</sup>H-NMR spectra have shown that  
10 there were 1.3 end groups of 2-hydroxyisopropyl per chain. It  
11 is suggested that significant termination by bimolecular  
12 combination occurred, between either a primary solvent  
13 radical and the propagating chains [Liu et al. Macromolecules  
14 35:1200-1207 2002].

15 US Patent 6,338,859 (Leroux et al.) discloses a class of  
16 poly(N-vinyl-2-pyrrolidone)-block-polyester copolymers. Such  
17 PVP block copolymers represent new biocompatible and  
18 degradable polymeric micellar systems which do not contain  
19 PEG, but which exhibit suitable properties as drug carriers.  
20 PVP shows remarkable diversity of interactions towards non-  
21 ionic and ionic cosolutes. Prior to the disclosure by Leroux  
22 et al., only a random graft copolymer, poly(N-vinyl-2-  
23 pyrrolidone)-graft-poly(L-lactide) had been described in the  
24 literature [Eguiburu et al. Polymer 37:3615-3622 1996].

1           In the synthesis of the amphiphilic diblock copolymer  
2 disclosed by Leroux *et al.* hydroxy-terminated PVP was  
3 prepared by radical polymerization using 2-isopropoxyethanol  
4 as a chain transfer agent. The block copolymer was obtained  
5 by anionic ring opening polymerization. Although the strategy  
6 of Leroux *et al.* works very well for the preparation of the  
7 desired amphiphilic diblock copolymers in the laboratory,  
8 several problems remain to be solved in order to achieve a  
9 scalable process. The use of crown ether and the need of  
10 dialysis and ultra-centrifugation for the copolymer  
11 purification are not desirable on an industrial scale.  
12 Furthermore, in the process disclosed by Leroux *et al.*, the  
13 degree of functionalization of hydroxyl-terminated PVP was  
14 not assessed.

15           What is lacking in the art is a process for preparing  
16 hydroxyl-terminated PVP, and using such functionalized PVP to  
17 prepare amphiphilic PVP-block-polyester block copolymers as  
18 well as other diblock or triblock copolymers consisting of  
19 PVP as one block; wherein the molecular weight,  
20 polydispersity index and functionality of the PVP can be  
21 controlled and wherein the process can be carried out on an  
22 industrial scale.

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## SUMMARY OF THE INVENTION

The instant invention provides a two-step polymerization process for preparing hydroxyl-terminated PVP and amphiphilic PVP-block-polyester as well as other diblock or triblock block copolymers consisting of PVP as one block. The process enables control of the molecular weight, polydispersity and functionality of the PVP. The diblock and triblock copolymers of the instant invention can be synthesized on an industrial scale for utilization in drug carrier systems.

The process of the instant invention comprises a two-step polymerization. The first step comprises free radical polymerization of VP in the presence of a radical initiator and an alcoholic solvent resulting in the synthesis of a low molecular weight PVP with a terminal hydroxyl group (PVP-OH). This step can be carried out with or without a chain transfer agent. The newly synthesized PVP-OH is purified by reprecipitation. The molecular weight of the PVP-OH can be effectively tuned and controlled by adjusting the molar ratios of radical initiator, chain transfer agent and alcohol to VP. With the use of higher concentrations, recombination of polymer chains is favored so that PVP with a hydroxyl group at both ends of each polymer chain (HO-PVP-OH) can be selectively obtained. Illustrative, albeit non-limiting examples of radical initiators are 2,2'-azobis(2-methyl-N-(2-hydroxyethyl)-propionamide (AMPAHE), 2,2'-azobis(2-methyl-N-



1 [2-(1-hydroxybutyl)]-propionamide and 1,1'-azobis(cyclohexane-  
2 carbonitrile). AMPAHE is a particularly preferred radical  
3 initiator, the use of which is illustrated in the examples  
4 herein. Illustrative, albeit non-limiting examples of  
5 alcoholic solvents are methanol, ethanol, isopropyl alcohol,  
6 n-propanol, n-butanol, tert-butanol, 1-pentanol and 2-  
7 pentanol. Isopropyl alcohol (IPA) is a particularly preferred  
8 alcoholic solvent, the use of which is illustrated in the  
9 examples herein. Illustrative, albeit non-limiting examples  
10 of chain transfer agents are 2-mercaptoethanol, 3-mercapto-1-  
11 propanol, 3-mercapto-2-propanol, 4-mercapto-1-butanol, 3-  
12 mercapto-2-butanol and 6-mercapto-1-hexanol. A particularly  
13 preferred chain transfer agent is 2-mercaptoethanol (MCE),  
14 the use of which is illustrated in the examples herein.

15 The second step of the process comprises anionic  
16 polymerization of a monomer or co-monomers using the dry  
17 hydroxyl-terminated PVP, synthesized in the first step, as a  
18 macroinitiator resulting in the formation of amphiphilic PVP-  
19 block-polyester diblock or triblock copolymers or other  
20 diblock and triblock copolymers consisting of PVP as one  
21 block. The second step is carried out using a catalyst or  
22 base in an inert aprotic solvent without the use of crown  
23 ether or other complexation agents. The newly formed block  
24 copolymers are isolated by precipitation and purified by

1 dissolution and re-precipitation. No dialysis is necessary  
2 for purification. Charcoal treatment can be used to remove  
3 any color from the newly formed block copolymers. The  
4 molecular weight of the block copolymer and the percentage  
5 content of polyester can be controlled by adjusting the ratio  
6 of the macroinitiator and the monomer(s). Illustrative,  
7 albeit non-limiting examples of catalysts are aluminium and  
8 tin alkoxides. Illustrative, albeit non-limiting examples of  
9 bases are potassium and sodium hydride. Illustrative, albeit  
10 non-limiting examples of inert aprotic solvents are  
11 tetrahydrofuran, toluene, diethyl ether and *tert*-butyl methyl  
12 ether. Tetrahydrofuran is a preferred inert aprotic solvent,  
13 the use of which is illustrated in the examples herein.

14 Accordingly, it is an objective of the instant invention  
15 to provide a two-step polymerization process for preparing  
16 PVP, amphiphilic PVP-block-polyester copolymers and other  
17 diblock or triblock copolymers consisting of PVP as one  
18 block.

19 It is a further objective of the instant invention to  
20 provide a two-step polymerization process for preparing  
21 diblock and triblock copolymers wherein said process enables  
22 control of the molecular weight, polydispersity and  
23 functionality of the components of each of the  
24 polymerizations.

1           It is yet another objective of the instant invention to  
2 provide a two-step polymerization process for preparing  
3 diblock and triblock copolymers wherein said process can be  
4 carried out on an industrial scale.

5  
6           It is a still further objective of the invention to  
7 provide (PVP)-block-polyester copolymers for use as drug  
8 carriers.

9           Other objects and advantages of this invention will  
10 become apparent from the following description taken in  
11 conjunction with the accompanying drawings wherein are set  
12 forth, by way of illustration and example, certain  
13 embodiments of this invention. The drawings constitute a  
14 part of this specification and include exemplary embodiments  
15 of the present invention and illustrate various objects and  
16 features thereof.

17  
18       **DEFINITIONS**

19           The following list defines terms, phrases and  
20 abbreviations used throughout the instant specification.  
21 Although the terms, phrases and abbreviations are listed in  
22 the singular tense the definitions are intended to encompass  
23 all grammatical forms.

24           As used herein, the abbreviation "PEG" refers to

1 poly(ethylene glycol).

2 As used herein, the abbreviation "PM" refers to  
3 polymeric micelles.

4 As used herein, the abbreviation "VP" refers to N-vinyl-  
5 2-pyrrolidone.

6 As used herein, the abbreviation "PVP" refers to poly(N-  
7 vinyl-2-pyrrolidone).

8 As used herein, the abbreviation "PVP-OH" refers to PVP  
9 with a hydroxyl group at one terminus of each polymer chain.

10 As used herein, the abbreviation "HO-PVP-OH" refers to  
11 PVP with hydroxyl groups at both termini of each polymer  
12 chain.

13 As used herein, the abbreviation "PDLLA" refers to  
14 poly(D,L-lactide).

15 As used herein, the abbreviation "PVP-*b*-PDLLA" refers to  
16 poly(N-vinylpyrrolidone)-*block*-poly(D,L-lactide).

17 As used herein, the abbreviation "MALDI-TOF" refers to  
18 matrix-assisted laser/desorption/ionization time-of-flight  
19 mass spectrometry.

20 As used herein, the abbreviation "MW" refers to  
21 molecular weight.

22 As used herein, the abbreviation " $M_w$ " refers to weight  
23 average molecular weight.

24 As used herein, the abbreviation " $M_n$ " refers to number-

1 average molecular weight.

2 As used herein, the abbreviation "NMR" refers to nuclear  
3 magnetic resonance.

4 As used herein, the abbreviation "EA" refers to  
5 elementary analysis.

6 As used herein, the abbreviation "SEC-LS" refers to  
7 size-exclusion chromatography coupled to light-scattering  
8 detection.

9 As used herein, the abbreviation "IPA" refers to  
10 isopropanol or isopropyl alcohol.

11 As used herein, the abbreviation "AMPAHE" refers to  
12 2,2'-azobis(2-methyl-N-(2-hydroxyethyl)-propionamide.

13 As used herein, the abbreviation "MCE" refers to 2-  
14 mercaptoethanol.

15 As used herein, the abbreviation "TBME" refers to *tert*-  
16 butyl methyl ether.

17 As used herein, the abbreviation "MIBK" refers to 4-  
18 methyl-2-pentanone.

19 As used herein, the abbreviation "THF" refers to  
20 tetrahydrofuran.

21 As used herein, the abbreviation "NaH" refers to sodium  
22 hydride.

23 As used herein, the abbreviation "LA" refers to D,L-  
24 lactide.

1 As used herein, the abbreviation "ATRP" refers to atom  
2 transfer radical polymerization.

3 As used herein, the abbreviation "DMF" refers to *N,N*-  
4 dimethylformamide.

5 As used herein, the abbreviation "TBA" refers to *tert*-  
6 butyl alcohol.

7 As used herein, the abbreviation "CAC" refers to  
8 critical association concentration.

9 As used herein, the abbreviation "DLS" refers to dynamic  
10 light scattering.

11 As used herein, the abbreviation "TGA" refers to  
12 thermogravimetry analysis.

13 As used herein, the abbreviation "CTA" refers to chain  
14 transfer agents.

15 As used herein, the abbreviation "PI" refers to  
16 polydispersity index.

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#### 18 BRIEF DESCRIPTION OF THE FIGURES

19 FIGURE 1 shows NMR data from example 1 ( $^1\text{H}$  NMR ( $\text{CDCl}_3$ ),  $\delta$   
20 (ppm). The product of step 1 is dried until the solvent peak  
21 disappears in NMR.

22 FIGURE 2 shows NMR data from example 2 ( $^1\text{H}$  NMR ( $\text{CDCl}_3$ ),  $\delta$   
23 (ppm). The product of step 2 is dried until the solvent peak  
24 disappears in NMR.

1        FIGURE 3 illustrates the synthesis of PVP-OH homopolymer  
2        (first polymerization) and PVP-*b*-PDLLA diblock copolymer  
3        (second polymerization).  
4

5        FIGURE 4 shows a spectrum resulting from MALDI-TOF  
6        spectrometry (example 8). MALDI-TOF analysis is useful for  
7        evaluation of the hydroxyl groups of PVP-OH.

8        FIGURES 5A-B show data evidencing the influence of the  
9        ratios of MCE (Figure 5A) and IPA (Figure 5B) to VP on the  $M_n$   
10       of PVP-OH.

11       FIGURE 6 shows a  $^1\text{H}$  NMR spectrum of PVP-OH-2500 in  $\text{CDCl}_3$   
12       (example 6).

13       FIGURES 7A-B show  $^1\text{H}$  NMR spectra of PVP-*b*-PDLLA  
14       (Diblock-47) in  $\text{CDCl}_3$  (Figure 7A) and in  $\text{D}_2\text{O}$  (Figure 7B).

15       FIGURE 8 shows a thermogravimetric profile of PVP-*b*-  
16       PDLLA diblock copolymer (Diblock-47).

17       FIGURE 9 shows the size distribution of micelles  
18       composed of PVP-*b*-PDLLA (Diblock-47) in water measured by  
19       DLS.

20       FIGURE 10 shows data for determination of CAC of PVP-*b*-  
21       PDLLA (Diblock-47) in water at  $25^\circ\text{C}$ .

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1     DETAILED DESCRIPTION OF THE INVENTION

2             The synthesis of the diblock and triblock copolymers is  
3     a two-step polymerization process.

4             The first step is a free radical polymerization of VP,  
5     carried out in an alcoholic solvent such as methanol,  
6     ethanol, isopropanol , n-propanol, n-butanol, 2-butanol,  
7     *tert*-butanol, 1-pentanol and 2-pentanol. Ideally, the boiling  
8     point of the solvent is in the vicinity of the cracking  
9     temperature of the radical initiator. Isopropanol (IPA) is a  
10    preferred solvent. The presence of a radical initiator is  
11    required. The radical initiator is selected from the group of  
12    azo derivatives comprising 2,2'-azobis(2-methyl-N-(2-  
13    hydroxyethyl)-propionamide) (AMPAHE), 2,2'-azobis{2-methyl-N-  
14    [2-(1-Hydroxybutyl)]propionamide and 1,1'-azobis(cyclohexane-  
15    carbonitrile). The preferred initiators are those having  
16    hydroxyl end groups, with 2,2'-azobis(2-methyl-N-(2-  
17    hydroxyethyl)-propionamide) (AMPAHE) being the most  
18    preferred. Thiol derivatives such as 2-mercaptoethanol, 3-  
19    mercapto-1-propanol, 3-mercapto-2-propanol, 4-mercapto-1-  
20    butanol, 3-mercapto-2-butanol and 6-mercapto-1-hexanol can be  
21    used as chain transfer agents. The preferred chain transfer  
22    agent is 2-mercaptoethanol (MCE). The molecular weight can be  
23    controlled by adjusting the molar ratios of MCE, AMPAHE and  
24    IPA to VP. The resulting first block homopolymer PVP can be



1 evaluated using techniques such as MALDI-TOF, SEC-LS, EA and  
2 NMR. PVP-OH is isolated by precipitation of its solution to  
3 an inert organic solvent with poor solubility for the  
4 polymer. The solvent or combination of solvents for  
5 dissolution is selected from the group comprising methanol,  
6 ethanol, IPA, acetone, 2-butanone, 4-methyl-2-pentanone,  
7 dichloromethane and tetrahydrofuran. The preferred solvents  
8 for dissolution are isopropanol and 4-methyl-2-pentanone, the  
9 use of which are illustrated in the examples herein. The  
10 inert organic solvent for precipitation is selected from the  
11 group comprising diethyl ether, *tert*-butyl methyl ether,  
12 hexane derivatives, heptane derivatives, ethyl acetate,  
13 isopropyl acetate, toluene and xylene derivatives. The  
14 preferred solvent for precipitation is *tert*-butyl methyl  
15 ether, the use of which is illustrated in the examples  
16 herein.

17 For the preparation of PVP-OH (first step of the  
18 process), once all reagents and solvent are charged, the  
19 reaction mixture is degassed prior to heating. The reaction  
20 temperature ranges from 60-140°C depending on the initiator  
21 and solvent chosen. In a preferred embodiment of the  
22 invention, a combination of IPA as solvent, AMPAHE as  
23 initiator and MCE as chain transfer agent is used and the  
24 reaction is carried out at reflux. The reaction time ranges

1 from 16 hours to 72 hours depending on the solvent, initiator  
2 and chain transfer agent. In the above preferred combination,  
3 a typical reaction time is between 30-48 hours.

4 It is important to ensure the dryness of the PVP-OH in  
5 order to succeed with the anionic ring opening polymerization  
6 in the next step. The drying of the polymer is performed  
7 using a vacuum oven with the temperature ramping towards  
8 110°C. Alternatively, further drying can be optionally  
9 performed using azeotropic distillation with an inert solvent  
10 such as toluene, xylene derivatives or heptane derivatives  
11 prior to the second polymerization.

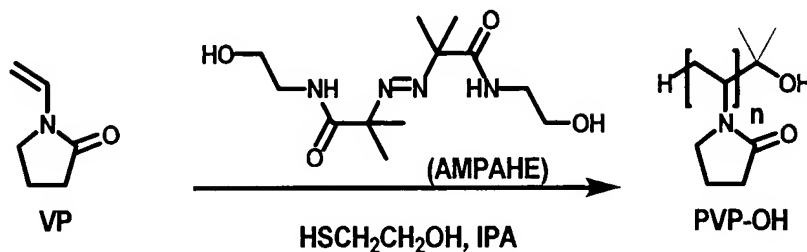
12 The second step is based on an anionic polymerization of  
13 cyclic ester, other cyclic lactone, methacrylate, or  
14 methacrylamide. This polymerization can be anionic via a  
15 macroinitiator or it can be catalyzed by aluminum or tin  
16 alkoxides. The macroinitiator is a metal PVP-hydroxylate  
17 obtained from the deprotonation of the terminal hydroxyl  
18 group with a metal hydride reagent such as sodium hydride or  
19 potassium hydride. The resulting second block is poly(ester)  
20 wherein the repeating unit is a lactide,  $\epsilon$ -caprolactone,  $\gamma$ -  
21 caprolactone or other cyclic ester. The resulting second  
22 block also can be poly(amino acid), polymethacrylate,  
23 polymethacrylamide or their copolymers. The blocks of  
24 homopolymers are linked chemically by a covalent bond. The

1 chemical linker between block homopolymers is a hydroxy  
2 derivative emerging from the radical initiator or chain  
3 transfer agent or an alcoholic solvent. An inert anhydrous  
4 aprotic solvent or combination of solvents such as  
5 tetrahydrofuran, toluene, diethyl ether, *tert*-butyl methyl  
6 ether can be used for the reaction, with tetrahydrofuran  
7 being preferred. The reaction temperature ranges from room  
8 temperature to about 70°C with preferred temperature being  
9 20-25°C. Upon completion of the reaction as evidenced by <sup>1</sup>H  
10 NMR (solvent peak disappears), the reaction mixture is  
11 filtered and the block copolymer is isolated from the  
12 filtrate by precipitation into an inert organic solvent which  
13 has poor solubility for the polymer. Similar solvent systems  
14 as for the precipitation of PVP-OH are used, with *tert*-butyl  
15 methyl ether being the most preferred solvent. Optionally,  
16 any color of PVP block copolymers can be removed by charcoal  
17 treatment and a white to off-white powder of the product is  
18 obtained.

19 The invention is further illustrated by the following non-  
20 limiting examples.

**Example 1** - Preparation of poly(N-vinyl-2-pyrrolidone) with a hydroxyl-bearing chain end (PVP-OH).

**SCHEME 1**



VP (200 g, 1.8 mol), AMPAHE (5.2 g, 0.018 mol) and MCE (5.0 mL, 0.072 mol) were dissolved in 3000 mL of IPA. The solution was degassed by nitrogen purge for 1 hour. The radical polymerization was carried out at reflux (about 89°C) with stirring under a dry nitrogen atmosphere for 44 hours. Then, after cooling to room temperature, most IPA was removed under reduced pressure and 400 mL of MIBK were added. Afterwards, the polymer was slowly precipitated into 5000 mL of TBME. The suspension was filtered. The filter cake was washed twice with 200 mL of TBME. The white powder thus obtained was purified by solubilization in 400 mL of MIBK and 100 mL of IPA and re-precipitation from 5000 mL of TBME. Finally, the product was

dried under vacuum (starting at room temperature then at 110°C, 1 torr) until disappearance of the solvent peak by NMR (Figure 1). The PVP-OH was obtained as a white powder: 122 g.  $M_n$ : 2060,  $M_w$ : 2600,  $M_w/M_n$ : 1.3.

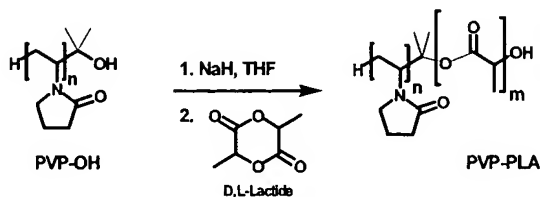
The instant inventors performed similar preparations of PVP-OH varying the different parameters such as the ratio of solvent/VP and the molar percentage of AMPAHE and MCE. Table 1 demonstrates that the molecular weight ( $M_w$ ) and number-average molecular weight ( $M_n$ ) of PVP-OH can be tuned effectively. The results showed also that the polydispersity index ( $M_w/M_n$ ) is generally lower when MCE is present. Lower  $M_w$  and  $M_n$  are obtained when the solvent/VP ratio is higher.

Table 1 Characterization of PVP-OH prepared under various conditions

Entry	VP (g)	AMPAHE (%mol)	MCE (%mol)	IPA/VP (volume ratio)	$M_n$ ( $\text{gmol}^{-1}$ )	$M_w$ ( $\text{gmol}^{-1}$ )	$M_w/M_n$
1	5	1.0	$\frac{3}{4}$	10	10290	21300	2.1
2	5	1.0	$\frac{3}{4}$	15	6760	15820	2.3
3	5	1.0	$\frac{3}{4}$	20	6300	12460	2.0
4	20	0.5	1.0	10	5100	11600	2.3
5	50	1.0	2.0	12	4000	6220	1.6
6	50	1.0	2.0	16	2510	3470	1.4
7	15	1.0	4.0	12	3230	4520	1.4
8	200	1.0	4.0	15	2060	2600	1.3
9	50	1.0	4.0	16	2170	3190	1.5

**Example 2** - Preparation of diblock copolymer poly(N-vinyl-2-pyrrolidone)-block-poly(DL-lactide) (PVP-PDLLA).

**Scheme 2**



PVP-OH (100 g, 48.5 mmol,  $M_n=2060$ ) was dissolved in 600 mL of anhydrous THF and sodium hydride 60 wt.% in mineral oil (3.0 g, 75 mmol) was added. The mixture was stirred for 30 minutes at room temperature and LA (125 g, 125% w/w) was then added. The anionic polymerization was carried out at room temperature with stirring under dry nitrogen atmosphere for 26 hours. Excess of sodium hydride was removed by filtration. The volume of filtrate was adjusted to 900 mL by addition of THF. Afterwards, the polymer solution was slowly precipitated into 4500 mL of TBME. The suspension was filtered. The filter cake was washed twice with 100 mL of TBME. The slightly yellow powder so obtained was purified by solubilization in 1215 mL of THF and 40.5 g of charcoal was added. The black suspension was stirred for 16 hours at room temperature then filtered over celite. The polymer was precipitated in 6000 mL of TBME. The suspension was filtered. The filter cake was washed twice with 100 mL of TBME and finally dried under

1 vacuum until disappearance of the solvent peak by NMR (Figure  
2 2). The PVP-PDLLA was obtained as a white to off-white  
3 powder: 62 g.  $M_n$ : 3140,  $M_w$ : 3445,  $M_w/M_n$  : 1.1.

4  
5 Empirical equations (Equation 1) and (Equation 2) were created  
6 to evaluate the molar percent PDLLA content by proton NMR and  
7 by Elemental Analysis, respectively.

8  
9 Equation 1: Determination of PDLLA (%mol) content by proton NMR

$$10 \quad \text{PLA (\%mol)} = \frac{I_{5.2 \text{ ppm}}}{\left[ \frac{(I_{4.5-0.8 \text{ ppm}}) - 3 \times I_{5.2 \text{ ppm}}}{9 \text{ H}} \right] + I_{5.2 \text{ ppm}}} \times 100 \quad (1)$$

14 Where  $I_{5.2 \text{ ppm}}$  represents the integration of the signal at 5.2 ppm  
15 which corresponds to the tertiary proton on C-10.  $I_{4.5-0.8 \text{ ppm}}$   
16 represents the integration of the signals of the protons of the  
17 PVP-OH. The contribution of the linker is omitted.

18  
19 Equation 2: Determination of PDLLA (%mol) content by Elemental  
20 Analysis (EA)

$$21 \quad \text{PLA (\%mol)} = \frac{7 \text{ C} - 36 \text{ N}}{7 \text{ C} - 18 \text{ N}} \times 100 \quad (2)$$

24 The block compositions of PVP and PDLLA correspond to the  
25 repeating unit of  $\text{C}_6\text{H}_9\text{NO}$  and  $\text{C}_3\text{H}_4\text{O}_2$ , respectively. The PDLLA

1 content (%mol) can be determined using equation (2) and based  
2 on the content of (c) and (N) atoms determined by EA .

3 Table 2 demonstrates the reproducibility of the molar percent  
4 PDLLA contents as well as the narrow polydispersity using the  
5 process.

6 Table 2 Preparation of PVP-PDLLA diblock copolymers according to  
7 Example 2.

8	Entry	M <sub>n</sub> PVP-OH used (gmol <sup>-1</sup> )	M <sub>n</sub> SEC (gmol <sup>-1</sup> )	M <sub>w</sub> SEC (gmol <sup>-1</sup> )	M <sub>w</sub> /M <sub>n</sub> SEC	PDLLA contents <sup>A</sup> (%mol)	PDLLA contents <sup>B</sup> (%mol)
9	1	2060	3140	3445	1.1	38	48
10	2	1850	3350	3690	1.1	38	48
11	3	2220	3680	4050	1.1	37	48

12 A: from equation 1, <sup>1</sup>H-NMR

13 B: from equation 2, EA ratio

14  
15 Table 3 demonstrates that the molar contents of PDLLA in the  
16 diblock copolymer are influenced by the weight ration of  
17 Lactide/PVP-OH charged to the reaction. A desired PDLLA%  
18 content can be predictably obtained.

19  
20 Table 3 Characterization of PVP-PDLLA diblock copolymers.

21	Entry	Lactide M <sub>n</sub> PVP-OH used used (%w/w) (gmol <sup>-1</sup> )	M <sub>n</sub> SEC (gmol <sup>-1</sup> )	M <sub>w</sub> SEC (gmol <sup>-1</sup> )	M <sub>w</sub> /M <sub>n</sub> SEC	PDLLA contents <sup>A</sup> (%mol)	PDLLA contents <sup>B</sup> (%mol)
22	1	90 2180	3145	4040	1.3	27	38
23	2	110 2165	3380	3720	1.1	35	42
24	3	125 2220	3680	4050	1.1	37	48

25 A: from equation 1, <sup>1</sup>H-NMR

26 B: from equation 2, EA ratio

27

28



1

2       **Example 3**-Synthesis of poly(N-vinylpyrrolidone) with a  
3 hydroxyl-bearing chain end (PVP-OH).

4       As shown in Figure 3, PVP-OH was synthesized by free  
5 radical polymerization of VP. VP (30 g, 270 mmol), AMPAHE  
6 (0.7783 g, 2.7 mmol) and MCE (0.844 g, 10.8 mmol) were  
7 dissolved in 540 mL of IPA. The solution was degassed with  
8 argon for 15 minutes. The polymerization was carried out at  
9 85°C for 24 hours. Then, most of IPA was removed under  
10 reduced pressure. Afterwards, the polymer was precipitated in  
11 about 300 mL of diethyl ether. The polymer was dissolved in  
12 60 mL of methylene chloride, and precipitated again in 300 mL  
13 of diethyl ether. Finally, the product (white powder) was  
14 transferred into a Whatman cellulose extraction thimble, and  
15 purified by diethyl ether Soxhlet extraction for 24 hours.  
16 The polymer was dried at 80°C under vacuum overnight.

17       **Example 4**-Synthesis of diblock copolymer poly  
18 (N-vinylpyrrolidone)-block-poly(D,L-lactide)

19       As illustrated in Figure 3, PVP-*b*-PDLLA was synthesized  
20 by anionic polymerization of LA using PVP-OH as  
21 macroinitiator. PVP-OH  $M_n$ : 2500 (15 g, 5.77 mmol) was  
22 dissolved in 250 mL toluene. Using a Dean-Stark trap, all  
23 products were dried with toluene as azeotropic solvent.  
24 Toluene was then removed by distillation under reduced

1 pressure. The polymer was dried under vacuum over  $P_2O_5$  at  
2 150°C for 4 hours. After cooling down to room temperature,  
3 potassium hydride (KH, 0.346 mg, 8.65 mmol) in mineral oil  
4 was added into the flask under argon atmosphere. The flask  
5 was placed under vacuum again for 30 minutes. A volume of 75  
6 mL freshly distilled and anhydrous THF was added to dissolve  
7 the mixture. After the polymer was dissolved, the solution  
8 was stirred for 10 minutes. LA (30 g, 20.8 mmol) and  
9 18-crown-6 (2.29 mg, 8.65 mmol), both previously dried under  
10 vacuum at 80°C for 4 hours, were placed in a flask and then,  
11 dissolved with a volume of 150 mL of anhydrous THF. The  
12 solution was transferred into the alcoholate solution under  
13 argon atmosphere, and stirred. The polymerization was  
14 carried out at 60°C for 18 hours. PVP-*b*-PDLLA was  
15 precipitated in 1.2 L of cold diethyl ether. The polymer was  
16 collected and dried under vacuum at room temperature. PVP-*b*-  
17 PDLLA (20 g) was dissolved in 100 mL of DMF. 100 mL of  
18 deionized water was added to the polymer solution for  
19 micellization. The micelle solution was placed in dialysis  
20 bag (Spectrum, MW cutoff: 3500) and dialyzed against water  
21 (8 L) at 4°C for 24 hours. Water was changed at least 4 times  
22 over that period. The aqueous solution was centrifuged at  
23 11600 g at 4 °C for 30 minutes, and then filtered through a  
24 0.2-µm filter. The filtered solution was collected and

1 freeze-dried during 48 hours. The diblock copolymer was  
2 stored at -80°C to avoid degradation.

3 **Example 5-Size-exclusion chromatography**

4 The SEC analysis was carried out on a Breeze Waters  
5 system using refractometer Waters 2410 (Milford,  
6 Massachusetts) and light-scattering (LS) detector Precision  
7 Detectors PD2000 (Bellingham, Massachusetts). LS data were  
8 collected at 15 and 90°. SEC was performed in DMF containing  
9 10 mM LiBr. 200 µL of solution (about 3%w/v) was injected  
10 through a series of 3 columns Styragel® Waters HT2, HT3 and  
11 HT4 at a flow rate of 1.0 mL/min, in order to separate MW  
12 ranging from 10<sup>2</sup> to 10<sup>6</sup>. The temperature of columns  
13 (separation) was maintained at 40°C, while the temperature of  
14 refractometer/LS detectors was set at 35°C. The instrument  
15 was calibrated with monodisperse polystyrene standards.

16 **Example 6-Nuclear magnetic resonance.**

17 <sup>1</sup>H- and <sup>13</sup>C-NMR spectra were recorded on Varian 300 and  
18 Bruker AMX 600 spectrometers (Milton, Ontario) in CDCl<sub>3</sub> at  
19 25°C. The PDLA content (%mol) was determined using equation  
20 1(as noted in Example 2). Where I<sub>5.2ppm</sub> represents to signal  
21 intensity at 5.2 ppm, and corresponds to the tertiary proton  
22 (α-position of carbonyl group). This signal was normalized to  
23 1. <sup>1</sup>H-NMR was also performed in deuteriated water (D<sub>2</sub>O) at  
24 25°C to evidence the presence of self-assembled micelle.

1  
2       **Example 7-Elementary Analysis**

3       EA was carried out in an oxidative atmosphere at 1021°C.  
4       Using a thermal conductivity probe, the amount of nitrogen  
5       oxide, carbonic acid, sulfur oxide (NO<sub>2</sub>, SO<sub>2</sub> and CO<sub>2</sub>) and  
6       water were quantified and provided the amount of nitrogen  
7       (N), carbon (C), hydrogen (H) and sulfur (S) atoms into the  
8       sample. The block compositions of PVP and PDLLA correspond to  
9       the repeating unit of C<sub>6</sub>H<sub>9</sub>NO and C<sub>3</sub>H<sub>4</sub>O<sub>2</sub>, respectively. The  
10      PDLLA content (%mol) was determined using equation 2 (as  
11      noted in Example 2) and based on the content of (C) and (N)  
12      atoms.

13       **Example 8-MALDI-TOF spectrometry for analysis of PVP**

14      MALDI-TOF mass spectra were obtained with a Micromass  
15      TofSpec-2E mass spectrometer (Manchester, UK). The instrument  
16      was operated in positive ion reflectron mode with an  
17      accelerating potential of +20 kV. Spectra were acquired by  
18      averaging at least 100 laser shots. Dithranol was used as a  
19      matrix and chloroform as a solvent. Sodium iodide was  
20      dissolved in methanol and used as the ionizing agent. Samples  
21      were prepared by mixing 20 µL of polymer solution (6-8 mg/mL)  
22      with 20 µL of matrix solution (10 mg/mL) and 10 µL of a  
23      solution of ionizing agent (2 mg/mL). Then 1 mL of these  
24      mixtures was deposited on a target plate and the solvent was

1 removed in a stream of nitrogen. An external multipoint  
2 calibration was performed by using bradykinin (1060.2 g/mol),  
3 angiotensin (1265.5 g/mol), substance P (1347.6 g/mol), renin  
4 substrate tetradecapeptide (1759.0 g/mol), and insulin  
5 (5733.5 g/mol) as standards.

6 **Example 9**-Viscosity-average molecular weight ( $M_v$ )  
7 determination of PVP.

8 The limiting viscosity number "K-value" (or Fikentscher  
9 K-value) of homopolymer PVP-OH was determined in accordance  
10 with BASF protocol (US Pharmacopoeia) using Ubbelohde  
11 viscometer Type 1a. With the K-value,  $M_v$ , is directly  
12 obtained from the following equation:  $M_v = 22.22(K + 0.075K^2)^{1.69}$

13 **Example 10**-Critical association concentration (CAC).

14 CAC was measured by the steady-state pyrene fluorescence  
15 method (Benahmed et al. Pharm. Res. 18:323-328 2001). The  
16 procedure is described briefly as follows. Several polymeric  
17 solutions in water containing  $10^{-7}M$  of pyrene were prepared  
18 and stirred overnight in the dark at 4°C. Steady-state  
19 fluorescent spectra were measured ( $\lambda_{ex}$ , = 390 nm) after 5  
20 minutes under stirring at 20°C using a Series 2 Aminco Bowman  
21 fluorimeter (Spectronic Instruments Inc., Rochester, NY).  
22 Experiments were run in duplicate.

23 **Example 11**-Dynamic light-scattering (DLS).

24 DLS was used for the determination of particle size in

1 water. For this analysis, a series of aqueous solutions of  
2 PVP-*b*-PDLLA with concentrations of 0.5, 1 and 2 mg/mL was  
3 prepared by dissolving the polymer directly in water. The  
4 solutions were analyzed with a Malvern instrument Autosizer  
5 4700 (Mississauga, Ontario). Each measurement was carried out  
6 in triplicate at 25°C at an angle of 90°C. The size  
7 distribution of particles and the intensity mean size were  
8 recorded.

9 **Example 12**-Thermogravimetry analysis (TGA).

10 TGA measurements were collected on a TA Instrument  
11 Hi-Res TGA 2950 Thermogravimetric Analyser (New Castle,  
12 Delaware).

13 About 1 mg of polymer was used for the experiments.  
14 Temperature ramp was 20°C/minutes between room temperature  
15 and 700°C. The residual amount of water was quantified after  
16 freeze-drying. PDLLA and PVP contents (%w/w) in diblock  
17 copolymer were also analyzed.

18 **Experimental Results From Examples**

19 Mercapto compounds are good chain transfer agents  
20 capable of functionalizing chain ends and controlling  
21 indirectly polymer molecular weight (Ranucci *et al.* Macromol.  
22 Chem. Phys. 196:763-774 1995; Ranucci *et al.* Macromol. Chem.  
23 Phys. 201:1219-1225 2000; Sanner *et al.* Proceedings of the  
24 International Symposium on Povidone; University of Kentucky:

1 Lexington, KY, page 20, 1983). A Hydroxyl group can be  
2 introduced at the end of polymer chains by using MCE as CTA  
3 in free radical polymerization of vinyl monomers. However, it  
4 was reported that when VP was radically polymerized in the  
5 presence of mercapto derivatives, only a small fraction of  
6 functionalized short oligomers was obtained. Moreover, a  
7 large amount of high MW polymers without terminal  
8 functionality was found in the product. This was due to the  
9 high transfer constant of thiol to VP (Ranucci *et al.*  
10 *Macromol. Chem. Phys.* 196:763-774 1995; Ranucci *et al.*  
11 *Macromol. Chem. Phys.* 201:1219-1225 2000). In the free  
12 radical polymerization of VP, radicals can transfer to  
13 solvent and possibly to a monomer. Hence, functionalized PVP  
14 had been synthesized using particular solvents (i.e.  
15 isopropoxyethanol). However, the functionality of PVP was not  
16 under control quantitatively (Ranucci *et al.* *Macromol. Chem.*  
17 *Phys.* 196:763-774 1995; Ranucci *et al.* *Macromol. Chem. Phys.*  
18 201:1219-1225 2000). In order to get quantitative  
19 hydroxyl-terminal PVP homopolymers and also to control their  
20 molecular weight profile, IPA, MCE and a hydroxyl-bearing azo  
21 initiator (AMPAHE) have been all combined in the instant  
22 invention for the radical polymerization of VP (see Figure  
23 3).

24 As shown in Figure 4, MALDI-TOF spectrometry showed that

the majority of PVP chains (>95%) bore a hydroxyl group at one chain end of PVP. Figure 4 shows a MALDI-TOF spectrum of PVP-OH-2500. Most chains featured a 2-hydroxyisopropyl group at the end, meaning that the solvent was the main specie initiating polymer growth. Using diluted conditions of polymerization, MALDI-TOF data suggests that no significant termination by bimolecular combination occurred during the reaction, because the mass of chain end was only that of IPA plus the sodium ion ( $59_{\text{IPA}} + 23_{\text{Na}^+} = 82$ , at  $n$  equals 0 in the linear equation). Two other distributions were also observed, which were attributed to PVP bearing MCE and VP as chain end, respectively. These distributions were only significant at low values of  $m/z$  ( $<1000 \text{ g mol}^{-1}$ ) and represented less than 5% of the spectrum, related to MCE- and VP-terminated chains. Since MCE is more efficient as a chain transfer agent than IPA, all the MCE were consumed early in the reaction. Previous syntheses of PVP in THF (instead of IPA) using MCE have shown that radicals may also transfer directly to monomers (Ranucci *et al.* Macromol. Chem. Phys. 196:763-774 1995; Ranucci *et al.* Macromol. Chem. Phys. 201:1219-1225 2000). In consequence, by combining MCE and IPA as CTA, the synthesis of low MW PVP could be achieved with the quantitative insertion of hydroxyl group on one chain end.

The molecular weights of PVP-OH were determined by SEC



1 and viscometry (Table 4). Polydispersity indexes (PI) of  
 2 about 1.5 indicated that radical transfers prevailed over  
 3 bimolecular combination, being consistent with MALDI-TOF  
 4 data. Results from SEC and viscometry were in good agreement.  
 5  $M_v$  might be slightly overestimated because the universal  
 6 equation established by BASF referred to a wide range of PVP  
 7 MW ( $10^3$  to  $10^6$ ). Mark-Houwink constants ( $K$  and  $\alpha$ ) of low MW  
 8 polymers differ from those having very high MW, which may  
 9 explain this overestimation. Analysis of PVP-OH by EA  
 10 revealed that the weight ratios of N/C atoms in all PVP-OH  
 11 were similar to the theoretical number (0.194).  
 12

13 Table 4. Characterization of hydroxyl-terminated PVP homopolymers.

Polymers	$M_n$	$M_w$	$M_w/M_n$	$M_v$	N/C
	SEC	SEC		Viscometer	
	(g mol <sup>-1</sup> )	(g mol <sup>-1</sup> )	SEC	(g mol <sup>-1</sup> )	EA
PVP-OH-2300	2300	3600	1.56	5400	0.192
PVP-OH-2500	2500	4000	1.60	5500	0.190
PVP-OH-4000	4000	7400	1.85	9000	0.193
PVP-OH-6100	6100	9600	1.57	11100	0.197

27 Molecular weight profile of PVP-OH was controlled by  
 28 changing ratios of both MCE (the CTA) and IPA, to VP monomer.  
 29 As expected, the molecular weights of PVP-OH decreased when  
 30

1 either CTA/VP or IPA/VP ratios increased (Figures 5A-B). In  
2 Figure 5A the ratios of IPA/VP are fixed at (■) 18 mL/g and (●)  
3 15mL/g. In Figure 5B the ratio of MCE/VP is fixed at (▲) 2.5%.

4 The  $^1\text{H}$  NMR spectrum of PVP-OH-2500 in  $\text{CDCl}_3$  is shown in  
5 Figure 6. The chemical shifts of the methylene groups of MCE  
6 are 2.7 and 3.8 ppm. When MCE was introduced at the end of the  
7 PVP-OH chains by forming S-C bond instead of S-H bond, the  
8 peaks of one methylene group appear at 2.7 and 2.75 ppm instead  
9 of 2.7 ppm, and the signal located around 3.8 ppm is overlapped  
10 with the peaks of PVP-OH in the spectrum. Signals between 1.1  
11 and 1.3 ppm are assigned to the methyl protons of the  
12 2-hydroxyisopropyl group (IPA fragment). These results suggest  
13 that PVP radicals transferred to both MCE and IPA, and this is  
14 in agreement with the results obtained from MALDI-TOF  
15 spectrometry.

16 Potassium hydroxylate derivatives are widely used for  
17 anionic ring-opening polymerization of LA (Nagasaki *et al.*  
18 *Macromolecules* 31:1473-1479 1998; Iijima *et al.* *Macromolecules*  
19 32:1140-1146 1999; Yasugi *et al.* *Macromolecules* 32:8024-8032  
20 1999). In the instant invention, the reaction between the OH  
21 group at the chain end of PVP-OH and potassium hydride produced  
22 potassium PVP-hydroxylate as macroinitiator for the  
23 polymerization of LA. Water and alcohol molecules in the  
24 reaction system may initiate the formation of free PDLLA

1 homopolymer. Since there are strong hydrogen bonds between PVP  
2 and water as well as alcohol, residues of these protic  
3 solvents, which interact with the polymer are difficult to  
4 remove (Haaf et al. Polymer J. 17:143-152 1985). In the present  
5 case, low MW PVP-OH were synthesized in IPA. Therefore, traces  
6 of IPA and water molecules might be contained in the polymer.  
7 Two drying steps were required for solvent removal. Briefly, at  
8 first, PVP-OH was dissolved in toluene and then, an azeotropic  
9 distillation was made. Then, the polymer was dried under vacuum  
10 at 150°C over P<sub>2</sub>O<sub>5</sub> for 4 hours. The polymer was actually molten  
11 under these conditions, and resulted in a highly dried  
12 material.

13 Molecular weight and PI of PVP-*b*-PDLLA were determined by  
14 SEC using light-scattering and a differential refractometer as  
15 detectors (Table 5). As expected, PVP-*b*-PDLLA MWs were larger  
16 than that of corresponding PVP-OH, while PI decreased. Anionic  
17 polymerization leads to very small PI (Nagasaki et al.  
18 Macromolecules 31:1473-1479 1998; Iijima et al. Macromolecules  
19 32:1140-1146 1999; Yasugi et al. Macromolecules 32:8024-8032  
20 1999). Therefore, the second polymerization step might decrease  
21 the PI of the diblock copolymer, suggesting that resulting  
22 materials were diblock copolymers and not a mixture of  
23 homopolymers. Another plausible explanation of lower PI was

1 that PVP-*b*-PDLLA having shortest PVP chains were removed by the  
2 precipitation in diethyl ether.

3 The PDLLA contents (%mol) in the diblock copolymers was  
4 determined by <sup>1</sup>H-NMR, EA and SEC. A <sup>1</sup>H-NMR spectrum of  
5 PVP-*b*-PDLLA (Diblock-47) copolymer in CDCl<sub>3</sub> is shown in Figure  
6 7A. The peak at 5.2 ppm corresponds to the -CH- group of PDLLA.  
7 Signals from 0.8 ppm to 4.5 ppm were assigned to all protons  
8 associated to PVP segment, which overlap the peak of PDLLA  
9 methyl group (1.4 ppm). PDLLA content was calculated using  
10 equation 1, and results are presented in Table 5. Since traces  
11 of water in PVP-*b*-PDLLA copolymers slightly overestimated the  
12 integration of PVP signals, EA was performed and the amount of  
13 nitrogen and carbon atoms were used for the calculation of  
14 PDLLA content using equation 2. As shown in equation 2 hydrogen  
15 atoms of moisture, even from the polymer, are not taken in  
16 account into the calculation of PDLLA content by EA. Contrary  
17 to <sup>1</sup>H-NMR analysis, EA results were quite constant and  
18 reproducible regardless of the moisture content. EA analysis  
19 turned out to be suitable for the quantification of PDLLA  
20 content into PVP-*b*-PDLLA. Actually, PDLLA content from NMR data  
21 was usually 6 to 8% less than that determined by EA. Although  
22 SEC resulted in higher PDLLA contents (about 5%) than EA, the  
23 consistence between EA, SEC and NMR were quite good (Table 5).

24

**Table 5.** Characterization of PVP-*b*-PDLLA diblock copolymers.

PVP- <i>b</i> -PDLLA <sup>A</sup>	PVP-OH used	M <sub>n</sub> SEC (g mol <sup>-1</sup> )	M <sub>w</sub> SEC (g mol <sup>-1</sup> )	M <sub>w</sub> /M <sub>n</sub> SEC	PDLLA NMR <sup>B</sup> %mol	PDLLA EA <sup>C</sup> %mol	PDLLA SEC <sup>D</sup> %mol
Diblock-47	PVP-OH-2500	4380	5000	1.14	38	47	54
Diblock-35	PVP-OH-2500	3840	5030	1.30	27	35	45
Diblock-37	PVP-OH-6100	8290	10360	1.39	32	37	36
Diblock-39	PVP-OH-4000	6070	8960	1.48	34	39	44
Diblock-45	PVP-OH-2300	3770	4860	1.29	37	45	50

A: labeling based on PDLLA content into PVP-*b*-PDLLA diblock copolymers, obtained from EA.

B: from equation 1

C: from equation 2

D: from the M<sub>n</sub> of PVP-OH and its corresponding PVP-*b*-PDLLA

Thermogravimetry (TGA) was also a good method for characterizing the diblock copolymer (Liggins et al. Adv. Drug Deliv. Rev. 54:191-202 2002). As shown in Figure 8, the trace of solvents (less than 4%) in the diblock polymer was removed below 100 °C. Figure 8 shows a thermogravimetric profile of PVP-*b*-PDLLA diblock copolymers (Diblock-47). PDLLA in the diblock copolymer was then degraded between 200 to 350

1 °C, followed by the degradation of PVP from 350 to 480 °C.  
2 Hence, the PDLLA content could also be determined by TGA. For  
3 instance, TGA of diblock-45 revealed a PDLLA content of  
4 48%mol, which was in good agreement with EA results.

5 Because of their amphiphilic properties, the well-  
6 defined PVP-*b*-PDLLA diblock copolymers can self-assemble in  
7 aqueous solution to form micelles. The size of micelles was  
8 measured by DLS at different concentrations. As shown in  
9 Figure 9, micelles composed of PVP-*b*-PDLLA (Diblock-47) in  
10 water at a concentration of 2 mg/mL feature a single narrow  
11 size distribution of about 40 nm. Figure 9 shows size  
12 distribution of micelles composed of PVP-*b*-PDLLA (Diblock-47)  
13 in water measured by DLS. Upon dilution towards 0.5 mg/mL, no  
14 change in the size of micelles was observed. The results  
15 indicate that there is no micelle aggregation in the  
16 solutions. In contrast, Benahmed et al. (C. Pharm. Res.  
17 18:323-328 2001) reported bimodal size distributions for  
18 PVP-*b*-PDLLA micelles. It has been suggested that the larger  
19 population reflects the aggregation of small individual  
20 micelles, governed by a secondary order of aggregation. The  
21 plausible explanation of the difference is that the molecular  
22 weights, PDLLA contents and polydispersity indices reported  
23 in Benahmed et al. were higher than the polymers described in  
24 the instant application.

1           Steady-state fluorescence, using pyrene as hydrophobic  
2   fluorescence probe, is well used as technique to show the  
3   formation of micelles (Zhao *et al.* Macromolecules 30:7143-  
4   7150 1997; Kabanov *et al.* Macromolecules 28:2303-2314 1995;  
5   Wilhelm *et al.* Macromolecules 24:1033-1040 1991). The  
6   polarity of the surrounding environment of the probe  
7   molecules affects some vibrational bands in the fluorescence  
8   emission spectrum. The changes in the relative intensity of  
9   the first and the third vibrational bands ( $I_{338}/I_{333}$ ), which is  
10   due to the shift of the (0,0) band from 333 to 338 nm in the  
11   emission spectrum have been suggested to examine the polarity  
12   of the microenvironment. The CAC of micelles can be  
13   determined by this method. After micellar formation, pyrene  
14   partitions into the micellar phase and the water phase. Since  
15   the core of the micelle is hydrophobic, the intensity ratio  
16   of  $I_{338}/I_{333}$  is changed. The extrapolation of tangent of the  
17   major change in the slope of the fluorescence intensity ratio  
18   leads to CAC. As illustrated in Figure 10, PVP-*b*-PDLLA  
19   copolymers exhibited a CAC of about 6 mg/L. Figure 10 shows  
20   the determination of CAC of PVP-*b*-PDLLA (Diblock 47) in water  
21   at 25°C.

22           The micellization of PVP-*b*-PDLLA also can be assessed by  
23   <sup>1</sup>H-NMR in D<sub>2</sub>O (Benahmed *et al.* C. Pharma. Res. 18:323-328  
24   2001; Yamamoto *et al.* J. Controlled Release 82:359-371 2002;

1 Heald *et al.* Langmuir 18:3669-3675 2002). Figure 7B shows an  
2  $^1\text{H}$ -NMR spectrum of PVP-*b*-PDLLA (Diblock-47) in  $\text{D}_2\text{O}$ . As is  
3 shown in Figure 7B, the peaks of the methyl protons ( $-\text{CH}_3$ )  
4 and the methine proton ( $\text{CH}-$ ) of PDLLA are highly suppressed  
5 while the peaks of PVP still appear in the spectrum,  
6 providing evidences of the formation of core-shell  
7 structures. The mobility of PDLLA chains in the core is  
8 highly restricted, resulting in masking of the PDLLA signals.  
9 On the other hand, PVP chains are still observed by  $^1\text{H}$ -NMR  
10 because of their high mobility as outer shell of micelles.

11 By combining MCE and IPA as chain transfer agents, PVP  
12 bearing one terminal hydroxyl group on one extremity was  
13 successfully synthesized by the first polymerization step of  
14 the process of the instant invention. PVP MWs were  
15 efficiently controlled by changing ratios of either MCE or  
16 IPA, to VP. Terminally functionalized low MW PVP were used to  
17 efficiently synthesize the PVP-*b*-PDLLA diblock copolymer by  
18 anionic ring-opening polymerization of D,L- lactide in the  
19 second polymerization step of the process of the instant  
20 invention. PVP-*b*-PDLLA self-assembled into micelles in water.  
21 These micelle-forming copolymers presented very low CAC of a  
22 few mg/L, leading to the formation of 40-nm polymeric  
23 micelles. These polymeric self-assemblies based on low  
24 molecular weight PVP blocks are useful as drug carriers for



1     parenteral administration.

2             All patents and publications mentioned in this  
3     specification are indicative of the levels of those skilled  
4     in the art to which the instant invention pertains. All  
5     patents and publications are herein incorporated by reference  
6     to the same extent as if each individual patent and  
7     publication was specifically and individually indicated to be  
8     incorporated by reference.

9             It is to be understood that while a certain form of the  
10    invention is illustrated, it is not to be limited to the  
11    specific form or arrangement of parts herein described and  
12    shown. It will be apparent to those skilled in the art that  
13    various changes may be made without departing from the scope  
14    of the invention and the invention is not to be considered  
15    limited to what is shown and described in the specification.

16            One skilled in the art will readily appreciate that the  
17    present invention is well adapted to carry out the objects  
18    and obtain the ends and advantages mentioned, as well as  
19    those inherent therein. The methods, procedures and  
20    techniques described herein are presently representative of  
21    the preferred embodiments, are intended to be exemplary and  
22    are not intended as limitations on the scope. Changes therein  
23    and other uses will occur to those skilled in the art which  
24    are encompassed within the spirit of the invention and are

1 defined by the scope of the appended claims. Although the  
2 invention has been described in connection with specific  
3 preferred embodiments, it should be understood that the  
4 invention as claimed should not be unduly limited to such  
5 specific embodiments. Indeed various modifications of the  
6 described modes for carrying out the invention which are  
7 obvious to those skilled in the art are intended to be within  
8 the scope of the following claims.

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